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COMPLETE SPECIFICATION.

Process for Refining of Vegetable Protein.

We, AJINOMOTO KABUSHIKI KAISHA, a Corporation organized under the laws of Japan, of No. 7, 1-chome, Takaracho, Chuoku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

10 This invention relates to a process for refining vegetable protein.

It is known that various vegetable seeds, for example, soybeans, peanuts, cotton seeds, rape-seeds, sesame seeds, kapok seeds and safflower seeds, contain considerable quantities of oil and protein, and the oil can be extracted and recovered from such seeds by pressing the seeds in a compressor or by extraction with organic solvents.

20 Such treated vegetable seeds from which at least some of the oil has been removed, generally contain substantial amounts of protein, and are used as protein-containing raw material in the chemical industry, the food industry and various other fields, an example of the use being in the manufacture of monosodium glutamate. However, these treated vegetable seeds contain various impurities such as ash and carbohydrates together with the protein, and it is often desirable to remove these impurities, as far as is possible, in order to increase the purity of the protein before using it as protein-containing raw material.

35 Several proposals have already been put forward for refining the protein of the treated vegetable seeds, and among them, the process most generally employed is a solid-liquid extraction system wherein the impurities are extracted and removed by using a suitable medium which may be diluted mineral acid or water-lower alcohol

mixture. However, when diluted mineral acid is used as the extracting medium, a considerable amount of the protein is dissolved in the medium and lost, thereby lowering the yield of protein obtained from the vegetable seeds, although the refined protein is of relatively high purity. On the other hand, when a water-lower alcohol mixture is used to refine the protein, the loss of protein due to dissolution in the extracting medium is relatively low, but the removal of impurities is not complete, with the result that the degree of purity of the protein obtained is relatively low. Moreover, the recovery of the lower alcohol from the protein separated from the impurities requires a very complicated procedure. In short, the known methods are not satisfactory from the industrial and practical viewpoint for refining the protein from the treated vegetable seeds.

The object of the present invention is to obtain highly refined protein from treated vegetable seeds, in high yield by a simple operation.

According to the present invention, there is provided a process for refining protein from vegetable seeds from which at least some of the oil has been removed, which process comprises treating the vegetable seeds with water in the presence of a cation exchange resin to extract the impurities therefrom. By the term "impurities" is meant extractable substances in the vegetable seeds other than the protein, and includes carbohydrates and ash (including sodium salts and potassium salts).

The protein can be obtained pure in solution by treatment with alkali, after which addition of a neutralizing agent to adjust the pH of the solution to the value of the isoelectric point of the protein, will cause the precipitation of the protein in very high purity, which precipitated protein may then

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be conventionally removed from the solution.

When the treated vegetable seeds are extracted with water in the presence of a cation exchange resin, water soluble components of the seeds, such as carbohydrates, ash, and a part of the protein, gradually dissolve in the water. However, as a result of the presence of the cation exchange resin, the cations of the ash content of the extraction medium are adsorbed by the cation exchange resin to reduce the content of ash in the medium. The decrease of the content of the cations of the ash causes a lowering of the pH value of the extraction medium to the order of 3 to 4 which is near to the value of the pH at the isoelectric point of the vegetable protein, so that the solubility of the protein becomes extremely low during the period that the ash content is reduced, which minimizes the loss of protein since a smaller portion remains dissolved in the extraction medium.

The invention will now be illustrated by taking the example of oil-removed soybeans which are a representative example of oil-removed vegetable seeds contemplated by the process of the present invention.

An extraction vessel was charged with 1 K.g. of oil-removed soybeans and 5 litres of water, and then 0.7 litre of Diaion SK No. 1 (H type of the product prepared by Mitsubishi Kasei-Kogyo K.K.) contained in a bag of synthetic fibre was introduced into the vessel. The mixture was agitated at room temperature for 30 minutes to cause the extraction of vegetable protein to take place, and the bag containing Diaion SK No. 1 was then raised from the mixture. The liquid extract was separated from the soybean residue by centrifuging.

The results of the above treatment is compared with the results of treatments employing conventional processes in the following table:—

TABLE 1.

	Treatment	Percentage loss of dissolved protein	Percentage extraction of carbohydrates	Percentage extraction of ash
50	In accordance with the above example	0.8 — 1.2	45 — 50	80 — 90
	Extraction with 0.1N HCl.	4 — 5	45 — 50	50 — 60
55	Extraction with 60% aqueous solution of methanol	2 — 3	35 — 40	20 — 30

In the case of extraction with 0.1N HCl, the treatment was carried out using oil-removed soybeans 1 K.g. to 0.1N HCl 5 litres, at room temperature for 30 minutes.

In the case of extraction with 60% aqueous solution of methanol, the treatment was carried out using oil-removed soybeans 1 K.g. to 60% methanol solution 5 litres at room temperature for 30 minutes.

From the above table, it can be seen that carbohydrates and ash are effectively extracted while the loss of dissolved protein is relatively small in the treatment according to the present invention. Thus it can be seen that both the purity and the yield of refined protein are increased by the process of the present invention.

Another advantage of the present invention is that inorganic salts and saccharified liquor can be easily recovered at relatively high purity as by-products. The cation exchange resin used in the extraction liquid and having adsorbed cations is treated with a mineral acid according to conventional methods to elute the adsorbed materials. The eluate is then concentrated and cooled

to obtain almost pure alkali salts, e.g. potassium salts. The cation exchange resin may be used repeatedly after its having been thoroughly washed with water. The liquid extract separated from vegetable seed residue contains substantially no inorganic salts and has very little protein dissolved therein, the major portion of the solute being sugars, which amount to 2—3 g/dl. Accordingly this liquid extract, with or without concentration, may be used as saccharified liquor (i.e. a source of saccharides).

In the process according to the present invention, any resin which can decompose neutral salts and adsorb their cations may be used as the cation exchange resin, and cation exchange resins having a sulfonic acid radical as the main functional group thereof are particularly advantageous, examples of such resins being Amberlite IR-120, Dowex 50, Duolite C-25, Diaion SK No. 1 and Zeolex SA. Other cation exchange resins of a complex type having a sulfonic acid radical as the main functional group thereof, such as for example, Amberlite XE-111, and cation exchange resins having

a phosphoric acid radical as the main functional group thereof, such as for example, Duolite C-61 and Permutit XP may also be used. The words "Amberlite", "Dowex", "Zeolux" and "Permutit" are Registered Trade Marks.

In order to obtain the best results, the amount of cation exchange resin used in the process according to the present invention should be more than 0.3 litre per K.g. of treated vegetable seed, and preferably the amount used should be from 0.6 to 0.8 litre per K.g. of the seed. The amount of water in the extraction medium, is preferably from 4 to 7 litres per K.g. of oil-removed vegetable seeds. However, the above amounts may be varied, depending on the particular case.

If the treated vegetable seeds and the cation exchange resin are allowed to mix, separation from each other will be very difficult. Accordingly, in the process of the present invention, suitable measure is generally taken to prevent such mixing. For example, the cation exchange resin may be put into a bag of synthetic textile fibre and thereafter immersed in the extraction medium. Alternatively two extraction vessels inter-connected by pipes may be used, the treated vegetable seeds being charged in to one vessel and the cation exchange resin being charged in to the other vessel, water being circulated between the two vessels.

The temperature at which the treatment is carried out has little effect on the efficiency of extraction.

The refined protein obtained by the process according to the present invention is of sufficiently high purity for many industrial purposes, but if it is desired to obtain a product of higher purity, the protein may be purified by treatment with dilute aqueous alkali solution and then with a neutralizing agent. When the oil-free vegetable seeds are treated with water in the presence of a cation exchange resin, and the residue obtained after separation from the extraction medium is treated with dilute aqueous alkali solution, an alkaline solution of protein containing substantially no impurities is obtained. A neutralizing agent is then added to the solution until the pH thereof approaches the pH value at the isoelectric

point of the protein thereby causing the protein to be precipitated. The precipitated protein is separated from the liquid to yield protein of extremely high purity.

It is a well known method to obtain pure protein by extracting protein from oil-free vegetable seeds with aqueous alkali solution and adjusting the pH of the aqueous alkaline protein solution obtained to the pH value at the isoelectric point of protein by adding acid, thereby precipitating protein, and then separating precipitated protein from the liquid present. However, the aqueous alkaline protein solution obtained by the conventional treatment with aqueous alkali solution contains considerable amounts of various impurities, since in addition to the protein, various other water soluble materials, such as the ash and carbohydrates, also dissolve into the extraction medium. When such a liquid is made acidic by the addition of acid, with a view to precipitating the protein, it is unavoidable that the impurities are also precipitated and become mixed with the precipitated protein. Under such conditions, the purity of the protein obtained is unavoidably reduced.

Furthermore, it is well known that the solubility of vegetable protein in water is increased by the presence of salts in the extraction medium. Hence, the solubility of the impure protein at the isoelectric point thereof will be increased, thereby lowering the yield of precipitated protein.

In contrast, in the process according to the present invention, the vegetable seeds from which at least some of the oil has been removed, to be treated with aqueous alkali solution have been subjected to treatment with water and a cation exchange resin, and water-soluble impurities, such as carbohydrates and ash, have already been removed from the seeds. Consequently the aqueous alkaline protein solution obtained in the method of the present invention is a solution of protein of high purity, and protein of high purity can be easily obtained in high yield from the solution.

In the following table, treatment according to the present invention is compared with a conventional extraction method employing alkali, in respect of the purity of protein obtained and the loss of protein:—

TABLE 2.

110	Purity of Protein (percentage of total nitrogen)	Percentage loss of Protein
Treatment in accordance with the present invention	15 — 16	2.2
Conventional alkali extraction	14 — 15	6.8

In the step of extracting the protein with aqueous dilute alkali solution according to the present invention, there is no specific limitation in regard to the alkali, the concentration, and the amount of alkali employed. However, it is preferable, in general, to use a solution of sodium hydroxide, potassium hydroxide, sodium carbonate or potassium carbonate, and with regard to the concentration, it is generally preferable to use a 0.1 to 1.0% alkali solution in an amount of 5 to 20 times the volume of the oil-removed seeds (dry volume) which have been subjected to the extraction of impurities with water and a cation exchange resin.

The recovery of protein from the aqueous alkaline solution obtained after treatment with alkali solution is carried out by adding acid, such as hydrochloric acid, sulphuric acid, citric acid or acetic acid, to adjust the pH of the solution to that at the isoelectric point of protein, thereby precipitating the protein. The precipitated protein is separated from the liquid by centrifuging or filtration, and dried to give pure protein.

If desired, before the addition of acid, the aqueous alkaline protein solution may be treated with a cation exchange resin (H type) to neutralize the liquid to pH 7.0, and thereafter the pH value may be adjusted to that at the isoelectric point of protein by adding the acid.

Pure protein material may also be obtained from the protein solution treated with a cation exchange resin to reduce the pH value of the solution to 7.0 as described above, by spray-drying the solution.

The initial condition of the different varieties of seeds depends upon the particular method employed for removing the oil from the seeds, that is whether a compression treatment, an extraction treatment, a low temperature treatment, or a high temperature treatment was employed, but the process according to the present invention can be applied to all conditions of seeds from which some of the oil has been removed, irrespective of the method used for removing the oil therefrom.

Furthermore, the invention is applicable to all varieties of vegetable seeds, that is to the residues of soybeans, peanuts, cottonseeds, rape seeds, sesame seeds, kapok seeds or safflower seeds after the extraction of at least some of the oil therefrom.

The treated vegetable seeds may be employed in any form, for example, as powder, flake or cake.

The following examples illustrate the invention:—

EXAMPLE 1.

1 kg. of oil-free soybeans (having the composition: total nitrogen 8.08%, carbohydrates 19.70%, ash 5.75%) was charged into an extraction vessel and 5.1 of water were

added. A bag made of "Saran" (a synthetic fibre textile, known under the Registered Trade Mark "Saran" and produced by Asahi-Dow Co.) containing 600 ml. of the cation exchange resin known under the Trade name "Zeolex SA" (H type) (a product of Zeolite Industrial Co., Ltd.) was immersed in the water and extraction was conducted at room temperature with agitation for 2 hours, after which the bag of Zeolex SA was removed from the extraction medium and the solid material remaining and the extraction liquid were separated.

The solid material (water content 64.2%) amounting to 1970 g. was dried and 705 g. of refined protein (total nitrogen content 11.30%) were obtained. The proportion of protein recovered was 98.7%. The filtrate amounting to 3770 ml. showed a pH value of 3.2 and had a composition including total nitrogen 0.022 g/dl., carbohydrates 2.42 g/dl. and potassium chloride 0.018 g/dl. This shows that the loss of protein to the solution was 1.03% and the efficiency of extraction of carbohydrates was 46.3%. On the other hand, the adsorbate was eluted from the Zeolex SA by 3N hydrochloric acid and the eluate was concentrated and cooled to recover inorganic salts comprising mainly potassium chloride. The proportion of ash extracted was about 82%.

EXAMPLE 2.

1 kg. of oil-free cotton seeds (having the composition: total nitrogen 7.33% carbohydrates 22.37%, ash 7.45%) was charged into an extraction vessel. A separate vessel was filled with 600 ml. the cation exchange resin Zeolex SA (H type). The two vessels were inter-connected by pipes and 5 l. of water was circulated between the two vessels by a pump, to carry out the extraction. After one hour, solid material and liquid extract were separated from each other and the solid material was dried to obtain 690 g. of refined protein (total nitrogen content 10.40%). The proportion of protein recovered was 97.8%, while the proportions of carbohydrates and ash extracted were 49% and 80%, respectively. The pH value of the liquid extract was 3.5. When an extraction of oil-free cotton seeds was conducted using water only with no cation exchange resin present, the rate of recovery of protein was only 89.2%.

EXAMPLE 3.

An extraction vessel was charged with 1 kg. of oil-free soybeans (having the composition: total nitrogen 8.13%, carbohydrates 20.3%, ash 5.80%) and 5 l. of water were added. A bag of "Saran" having similar contents to that employed in Example 1, was immersed in the water and extraction was carried out under agitation at room tempera-

ture for one hour, after which the bag was removed and the solid material and liquid extract remaining were separated by centrifuging. The proportion of carbohydrate extracted was 47%, the loss of protein was 1.23%, and the proportion of ash extracted was 85%, almost all having been adsorbed by the resin and recovered. The solid material amounted to 2,105 g. (water content 66.1%) and the total proportion of nitrogen in the dried material was 11.25%.

To this material, were added 7550 cc. of 0.35% aqueous caustic soda solution and after extracting protein for 20 hours at a pH value of 9.0, the mass was filtered. The residue from filtration was dried and 475 g. of dried material (total nitrogen content 10.42%) were obtained. The filtrate, an alkaline aqueous solution of protein amounted to 6320 cc. 400 ml. of Zeolux SA (H type) contained in a bag of "Saran" were immersed and shaken in the filtrate to neutralize and desalt it and the pH value of the filtrate was made 4.5. Precipitated protein was separated by centrifuging and dried to give 193 g. of pure protein (total nitrogen content 15.55%). 5470 cc. of waste liquor contained a total nitrogen content of 0.015 g/ml.

Accordingly the loss of protein from the oil-free soybean was 2.24% while the proportion of carbohydrates extracted was 60.4%.

EXAMPLE 4.

12 l. of 0.22% caustic soda solution were added to 2105 g. of solid material obtained by the procedure of Example 1, and extraction of protein was carried out at a pH value of 10.5 for 20 hours. The solid was separated by filtration, washed with water and dried, to give 304 g. of dried material (total nitrogen content 8.10%). 21 l. of an alkaline aqueous solution of protein was obtained. Into the solution was poured 500 ml. of Diaion SK No. 1 (H type) and the solution was stirred to adjust the pH there-

of to 7. After removing the resin by decantation, the pH of the solution was adjusted to 4.2 by the addition of hydrochloric acid. Precipitated protein was separated by centrifuging and dried. 353 g. of pure protein (total nitrogen content 15.25%) were obtained. The yield was 66%, and the loss of dissolved protein during the operation was 3.8%.

WHAT WE CLAIM IS:—

1. A process for refining protein from vegetable seeds from which at least some of the oil has been removed, which process comprises treating the vegetable seeds with water in the presence of a cation exchange resin to extract the impurities therefrom.
2. A process according to Claim 1 and further treating the protein residue with a solution of an alkali to obtain a purified protein solution, adding a neutralizing agent to said purified protein solution to adjust the pH thereof to that of the isoelectric point of the protein in order to precipitate protein of very high purity, and thereafter separating the precipitated protein of high purity from the solution.
3. A process for refining protein from vegetable seeds from which at least some of the oil has been removed, substantially as described in any one of the foregoing examples.
4. A process for recovering protein of high purity from vegetable seeds from which substantially all the oil has been removed, substantially as described in any one of the foregoing examples.
5. Vegetable protein, whenever obtained by a process claimed in any preceding claim.

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